Office Action Summary	Application No.	Applicant(s)	
	10/576,633	SEBASTIAN ET AL.	
	Examiner	Art Unit	
	OLUWATOSIN OGUNBIYI	1645	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 September 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *20091028*.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

CONTINUED EXAMINATION

A request for continued examination under 37 CFR 1.114 was filed in this application after appeal to the Board of Patent Appeals and Interferences, but prior to a decision on the appeal. Since this application is eligible for continued examination under 37 CFR 1.114 and the fee set forth in 37 CFR 1.17(e) has been timely paid, the appeal has been withdrawn pursuant to 37 CFR 1.114 and prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 9/8/09 has been entered.

Claims 1-12 are pending. Claims 1-12 are under examination.

Examiner initiated Interview

See substance of interview in interview summary attached.

Rejections Maintained

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The rejection of claims 1-12 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is maintained for the reasons below. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are drawn to a method for detecting the presence or absence of a bacterium in a sample selected from a wound, a body fluid, or fluid from a wound, said method comprising the steps of:

a) contacting said sample with a surface-attached detectably labeled synthetic alpha-1-proteinase inhibitor reactive site loop domain peptide substrate under conditions that result in cleavage of said substrate by an enzyme produced in said sample by a bacterium; and

b) detecting a cleavage or an absence of cleavage of the substrate, the cleavage of the substrate indicating the presence of the bacterium in the sample and absence of the cleavage of the substrate indicating absence of the bacterium in the sample.

The breadth of claims covers the detection of any bacterium in a wound, a body fluid, or fluid from a wound via the cleavage of synthetic alpha-1-proteinase inhibitor reactive site loop domain peptide substrate attached to a surface by *any enzyme* produced by said bacterium.

The specification contemplates the detection of the presence or absence of bacteria in a wound surfaces and body fluids (see p. 11 last bridging paragraph to p.12 lines 1-10). However, the instantly claimed method does not take into account that wound surfaces and body fluids comprise human proteases (Steffensen et al Crit Rev Oral Biol Med 12(5):373-398, 2001, Armstrong et al J Am Podiatr Med Assoc 92(1): 12-18, 1998 and Ungar et al J Exp Med. 1961 January 31; 113(2): 359–380) that may be confounding factors in the instant method of bacterium detection. The specification in fig. 1A teaches that metalloproteinases (MMP1, MMP8 etc) from bacteria such as *S. aureus* cleave the RSL peptide of alpha-1-proteinase inhibitor. However, host matrix metalloproteinases such as MMP8 play a role in wound healing and can be found in wound tissue. Even bacterial infection of a wound results in prolonged elevation of proinflammatory cytokines which in turn causes increases in levels of matrix metalloproteinases released from neutrophils and macrophages (Cullen et al WO 03/040406 A2, 2003, p. 1 lines 24-28, p. 3 lines 8-15). The instant method as claimed does not control for cleavage of the RSL domain peptide by non-bacterial enzymes or

proteases that may be present in wound surfaces and body fluids because the same type of bacterial enzymes that cleave RSL domain of alpha-1-proteinase inhibitor is present in wound surface. For example, since host matrix metalloproteinases e.g. MMP8, MMP1 are present in wound tissue and detection of cleavage of RSL by these host enzymes will not correctly indicate that bacteria is present in said wounds. Desrochers et al (J. Clin. Invest. 1991 88:2258-2265, see whole document especially fig.5) teaches that human MMP1 cleaves the RSL domain of alpha-1-proteinase inhibitor. The instantly claimed method does not distinguish between modification of RSL domain peptides by proteases produced bacteria and by host proteases present in wound surfaces and body fluids.

Example 2 p. 18-19, in the specification teaches that wound dressings obtained from patients (no information on the wounds or patients was obtained) were extracted in PBS overnight and cleavage reaction was carried out on these samples with an RSL domain peptide of alpha-1-proteinase inhibitor. Figures 10A-D present the results from this assay and the figure legend states that the graphs illustrate the relative fluorescence of bacteria extracted from wound dressings. Since wound surfaces contain host enzymes that can cleave the detectably labeled RSL domain peptide it is not certain that the fluorescence observed is due to enzymes produced by bacteria. No information on the wounds or patients was obtained and the samples were not cultured to determine that they were infected with bacteria. Desrochers teaches that matrix metalloproteinase 1 (MMP1) is synthesized in epithelial cells which can be found in wounds in response to proinflammatory cytokines (p. 2258 column 2) which in turn can be induced by the presence of bacteria (See. Xue et al Clinical and Experimental Ophthalmology vol. 28 issue 3 p. 197-200, 12/25/01). Thus, the same bacterial enzyme e.g. MMP1 that can be produced by bacteria that cleaves RSL domain of alpha-1-proteinase inhibitor (see drawings fig. 1A) is also produced i.e. MMP1 by the body in response to bacterial infection.

As to the requirement of the claims that any enzyme produced by a bacterium can cleave the synthetic alpha-1-proteinase inhibitor RSL domain peptide, the specification teaches that secreted proteases cleave alpha-1-proteinase inhibitor RSL

domain peptide(s) (see for example, p.20 lines 10-14). Without further guidance, it is unpredictable as to which other enzymes apart from proteases can cleave alpha-1-proteinase inhibitor RSL domain peptide(s).

Applicants' arguments and the response:

The declaration of Dr. Mitchell C. Sanders under 37 CFR 1.132 filed 9/8/09 has been fully considered and is insufficient to overcome the rejection of claims 1-12 based upon 35 U.S.C. 112, first paragraph as set forth above because there is no showing that the objective evidence of nonobviousness is commensurate in scope with the claims. See MPEP § 716.

The declaration states that the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor ([it is noted here that the claims recite alpha 1-proteinase inhibitor not alpha 1 trypsin inhibitor])that is placed between an anchor (for example, a 60 µM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag). A dressing sensor consisting of a polyurethane foam bottom layer and the CPI2S RSL peptide-blue bead chemistry and at the top membrane is also depicted in the poster submitted with the instant declaration, presented at April 26-29, 2009 annual meeting of the Society for Advanced Wound Care. The declaration states that the steric hindrance of labeling a small peptide with very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus makes it virtually inaccessible to host proteases that are typically much larger 30 K states that counter to the findings described in the Desrocher et al . publication which demonstrated reactivity of MMPs with RSL, "we have unequivocally demonstrated that the RSL sensor in the context of the dressing diagnostic can be quite specific for bacterial proteases". The declaration states that such a sensor in this context does not cross-react with proteases from blood or human fluid because of the spatial representation of the peptide in the context of a bead anchor and aromatic leaving group (blue dye or biotin/polyhistidine).

The evidence as set forth above in the declaration is not commensurate with the scope of the claims because the evidence in the declaration is drawn to the use of small

(10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 µM Tric Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag). Claim 1 does not use a 10 amino acid RSL peptide substrate. In addition, even though the claims recited that the detectably labeled RSL peptide substrate is surface attached, the claims do not indicate that the attachment is made to a bead of a particular size and that the label is a blue dye #1 or biotin/polyhistidine tag. It is the steric hindrance of labeling the small peptide with a very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus that makes it virtually impossible for host proteases to cleave the RSL small peptide. This structure of a very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus is not recited in the claims.

Figure 1 of the declaration states that experiments run *in vitro* indicate that a fragment of RSL i.e. peptide CPI2S does not cross react with MMPs 1,2, 9 or 13 or physiological levels of human neutrophil elastase. The declaration does not set forth the peptide sequence of "CPI2S" and whether it has the same sequence as the peptide named CPI2 which is SEQ ID NO: 2 of the instant specification. The proposed publication titled "Rapid Measurement of Protease Activity Prevalent in Bacteria from Wounds: A diagnostic for Total Bioburdern: submitted along with the instant declaration teaches that a peptide named "CPI2S" has the sequence GMAFLEAIPC (see p. 5 of 46) which is different from the sequence of CPI2 (SEQ ID NO: 2 which has the sequence EGAMFLEAIPMSIPK). The publication states that the CPI2S peptide attached to a bead has reduced sensitivity to human neutrophil elastase (p. 14 of 46 first paragraph, p. 19 of 46 second paragraph, figure 7 legend p. 36 of 46). However, the CPI2 (SEQ ID NO: 2) peptide attached to bead was clipped by human neutrophil elastase. This CPI2S" having the sequence GMAFLEAIPC is not disclosed in the claims or the specification.

Furthermore, it is not clear whether the experiment in figure 1of the declaration is carried out with the CPI2S attached to bead on the amino terminus and a label on the C terminus. Tthe proposed publication tested the sensitivity of CPIS attached to bead to

human proteases and found reduced sensitivity of the CPIS-bead to human neutrophil elastase and MMP2 and MMP9. CPI2 (SEQ ID NO: 2) attached to a bead was cleaved by human neutrophil elastase but not MMP2 and MMP9. See p. 14 of 46 first paragraph, p. 19 of 46 second paragraph, and figure 7 legend p. 36 of 46). Thus, attachment to bead of particular size plays an important role but even with the bead, CPI2 (SEQ ID NO: 2) attached to a bead was still cleaved by human neutrophil elastase.

Also, claim 1 is not limited to peptide "CPI2S" or limited to SEQ ID NO: 2. Any reactive site loop domain peptide can be used for the instantly claimed method. As evidenced, by the specification the RSL peptide SEQ ID NO: 4 has cleavage sites for human metalloproteinases MMP-1,3,7,8, and 9 (see figure 1A). Further, SEQ ID NO: 2 has cleavage sites for human metalloproteinases not tested such as MMP8, MMP3 and MMP7 (see specification figure 1A). The experiment set forth in Figure 1 of the declaration does not provide positive controls of the reaction between the MMP tested and their known substrates as a comparison with the reaction of the MMPs with the CPI2 peptide tested.

The evidence provided is not commensurate with the scope of the claims as set forth above. In addition, the art clearly teaches (Desrochers et al. J. Clin. Invest. 1991 88:2258-2265, see whole document especially fig.5) that human MMP1 cleaves the RSL domain of alpha-1-proteinase inhibitor and the specification in Fig. 1 A also corroborates the fact that several human MMPs cleave an RSL peptide substrate. Nelson et al (Analytical Biochemistry 260:230-236 199, cited in IDS) also teaches the proteolytic cleavage of an RSL sequence for alpha 1 proteinase inhibitor by human MMP1, 8 and 9 (see table 2 of Nelson et al).

The instant method is detecting the presence or absence of a bacterium directly in samples such as wound, body fluid or fluid from a wound. The proposed publication submitted along with the declaration selectively removes the host enzyme, human neutrophil elastase (HNE) from samples obtained from wounds of patients (see p. 12 of 46). Some wound samples contained high human elastase concentrations high enough to match bacterial protease activity in terms of interaction with a CPI2 (SEQ ID NO: 2)

substrate (figure 7 of proposed publication and figure 7 legend on p. 36 of 46) and additional methods (e.g. cation exchange chromatography) were used to remove HNE from the diagnostic test so it does not interfere from the assay (see p.21 of 46). The instant claims do not remove human enzymes from the samples listed that would interfere with the instant method of detection of the presence or absence of bacteria. In the discussion section of the proposed publication submitted with the declaration, p. 26 of 46 teaches that since elevated HNE could cause protease activity to be detected in the absence of bacterial proteases, CPI2 (SEQ ID NO: 2) was altered to reduce cleavage by HNE relative to bacterial proteases. No such alteration of SEQ ID NO: 2 is disclosed in the claims or the specification.

In view of the above considerations including the attachment of the peptide to beads of particular size not disclosed in the claims, the interference of human proteases in the instant method, the cleavage of CPI2 peptide(SEQ ID NO: 2) by human neutrophil elastases despite being attached to a bead, evidence in the art for cleavage of the RSL peptide by human proteases in the specification (figure 1) and in the art (as cited above), the evidence in the submitted proposed publication; the declaration and the documents submitted along with are inefficient to overcome the instant rejection.

Status of Claims

Claims 1-12 are rejected. No claims allowed.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to OLUWATOSIN OGUNBIYI whose telephone number is 571-272-9939. The examiner can normally be reached on M-F 8:30 am- 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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